ASSESSMENT OF A BIOLOGICAL APPROACH FOR THE PROTECTION OF COPPER ALLOYS ARTEFACTS

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Abstract

We propose an innovative treatment by which the protection of copper-based artefacts can be provided by naturally occurring micro-organisms. The properties of some fungi were exploited for the transformation of existing corrosion patinas into copper oxalates. The latter are known to be insoluble and chemically stable.

Within the earlier EU-ARTECH and BAHAMAS projects, very promising results were obtained with an almost 100% conversion from copper hydroxysulfates and hydroxychlorides into copper oxalates. A fungal strain was used which had been isolated from vineyard soil heavily contaminated with copper. Further scientific investigations were carried out to determine the parameters of the process and allowing the formation of a reproducible and homogeneous patina of copper oxalates, called biopatina to highlight its biological origin. Particular attention was paid to the efficacy, durability and impact on colour of the newly developed treatment.

Different copper and bronze coupons with either urban or marine patinas were prepared. Several analytical techniques were used for the characterisation of the patinas: Fourier Transform InfraRed microspectroscopy (µFTIR), colourimetry and Electrochemical Impedance Spectroscopy (EIS). The coupons were treated with either the biological treatment or reference materials (wax: Cosmoloid H80; silane: Dynasylan® F8263) and exposed to atmospheric corrosion (ISMAR-SMS Genoa Harbour, corrosivity class 5) in December 2011. The long-term behaviour and performance of the treatments under study was monitored and compared over a one year period using the same complement of analytical techniques used for the characterisation of the original patinas.

These first measurements suggested a different weathering behaviour of the biopatina. In fact, in comparison to the silane and wax treatments the biopatina showed a lower colour variation, and a corrosion stabilisation process seemed to be in progress. A deeper analysis of colour and corrosion rate variation from different application methods was also achieved. The complete assessment of the different treatments will be finished this year.

Keywords
Protective treatments, corrosion, metal artefacts, copper, bronze, fungi.

Research aims
The research activity set out to develop an alternative biological treatment for the protection of copper-based artefacts. Particular attention was devoted to the efficacy, compatibility and impact on colour of the developed treatment, to overcome the problems associated with the treatments currently in use. To achieve these objectives, some fungal species able to transform metal compounds into metal oxalates at ambient temperature and pressure were exploited. This use of biological treatments would represent an eco-friendly strategy with little or no adverse effect on health or on the environment. In addition, green copper oxalates present some advantages in providing a surface with good protection: compatibility, insolubility and chemical stability. Most of all, they are not part of the phenomenon of cyclical corrosion. The research work investigated their long-term behaviour under aggressive corrosion conditions, as well as the parameters allowing the formation of a reproducible and homogeneous copper-oxalates patina.

Introduction
Archaeological and artistic metal artefacts encounter irreversible changes in their original appearance and structure due to electrochemical processes, chemical reactions with pollutants, and the physical phenomenon of deposit accumulation. These alterations are most often unstable and constant efforts are devoted to overcome this continuous damage with different interventions of conservation and restoration, ensuring the preservation of the aesthetic and historical value of the original artwork. The most commonly used technique is the treatment of the surface with waxes, either alone or as sacrificial topcoat on acrylic lacquers (Marabelli and Napolitano, 1991; Letardi, 2004). Among acrylic resins, Incralac™ has been also widely applied (Brostoff, 2003). Inhibitors, such as benzotriazole, are quite efficient as primer when coated with acrylic lacquers (Brostoff, 2003). However, all these protective systems require periodic maintenance due to their poor long-term performance (Dillmann, 2007; Argyropoulos, 2008; Watkinson, 2010). Such maintenance programmes can prove difficult to achieve as they are dependent on the allocation of ever-reducing funds.

At present, there is a pressing need, as expressed by art conservators and restorers, to develop novel conservation methods compatible with the object's natural patina. In fact, the nature of the corrosion products present on the surface of artistic and archaeological metal artefacts is intrinsically related to the environmental context (atmospheric or burial). Therefore, in order to protect and inhibit the corrosion of such metal objects effectively, the practices adopted should take into account the nature of the patina and its corrosion behaviour. Alternative means that would improve protective systems consist of modifying existing corrosion products and creating more stable and less soluble compounds while maintaining the surface's physical appearance.

The ability of some species of fungi to transform metal compounds into metal oxalates has been reported in the literature (Sayer and Gadd, 1997; Gharieb, et al,. 2004). This capability has been widely exploited to immobilise toxic metals in the environment and in waste treatment (Hazen and Tabak, 2005; Godlewska-Żyłkiewicz, 2006). In the field of the conservation and restoration of metal artefacts, the presence of copper oxalates on outdoor exposed bronzes has already been identified, but is not associated with the phenomenon of cyclical corrosion (Nassau et al., 1987). Instead, compact patinas of an attractive green colour are created on the bronze surface. With a high degree of insolubility and chemical stability even in an acidic environment (pH 3), it provides the surface with good protection (Marabelli and Mazzeo, 1993). There is a growing interest in the synthesis of inorganic materials by biological systems. This approach is more environmentally friendly as it functions close to ambient temperature and pressure and does not require the use of toxic materials (Bharde et al., 2006). Some conservation methods already have successfully used micro-organisms in the protection of stone monuments by inducing carbonate mineralisation (Castanier et al., 2000) or removing black crusts (Cappitelli et al., 2007). In corrosion control, the use of microbial films has improved significantly within the last years, and therefore represents a novel strategy for protecting metal substrates (Zuo, 2007).

Within the EU-ARTECH project (6th EU-Framework Program, 2004-2009), a biological treatment using fungi resistant to copper was developed for the treatment of outdoor bronze monuments (Di Francesco et al., 2007; Joseph et al., 2007; Mazzeo et al., 2008). A strain of *Beauveria bassiana* was isolated by the Laboratory of Microbiology at the University of Neuchâtel from vineyard soil highly contaminated with copper. It showed the best performance with almost a 100% conversion of copper hydroxysulphates and hydroxychlorides into copper oxalates. In the BAHAMAS project which followed (7th EU-Framework Programme, Marie-Curie Action, 2010-2012), the formation mechanisms and adhesion properties of metal oxalates on different metal substrates (copper, iron and silver) were investigated. The crystals aggregates were characterised through ESEM, FTIR and Raman microscopies, either on metal-enriched media or on corroded coupons (Joseph et al., 2011; 2012a; b). Different coupons were also treated with *B. bassiana* or reference materials (e.g. wax: Cosmoloid H80; silane: Dynasylan® F8263) and exposed in the CNR-ISMAR (Genoa, Italy) SMS experimental marine station (corrosivity class 5). The treatments' performances were compared and monitored over a six months period. In order to better evaluate the long-term performance of the treatments under study, the monitoring was extended beyond project conclusion to last 18 months. In parallel, optimisation tests of the biological treatment were also performed. In particular, different application methods were evaluated based on their colour and corrosion rate variation. This assessment and the results of the ageing so far achieved are presented in this contribution.

Materials and methods

Samples

Twelve bronze (58 x 58 x 3 mm) square samples QQ were cut from cast ingots with the composition Cu 90%, Sn 8%, Pb 2%, then polished using silicon carbide (SiC) metallographic grinding paper 1200 grit in water, washed in ethanol and then dried in air. The natural patina was made up by exposure on a rack in the urban marine environment at the ISMAR SMS site in Genoa Harbour from May 2007 to October 2009 (30 months). Orientation and position according to ISO 9223 standard were selected: the samples were facing south exposed skyward in the position of 45 degrees from the horizontal. The patina developed is mainly composed of cuprite Cu_2O and $Cu_2Cl(OH)_3$ isomorphs (Atacamite, Clinoacamite), with traces of brochantite $Cu_4SO_4(OH)_6$.

Twelve copper (50 x 50 x 1 mm) coupons were cut from a naturally aged copper roof in Zürich. All copper urban natural (CUN) coupons exhibit a typical urban natural patina mainly composed of a copper hydroxysulfate brochantite and cuprite.

For each group, three samples were used for each treatment to comply with minimal statistical requirements. Before and after treatment, all coupons were documented with a 600 dpi scanner. Along with coupons undergoing the biological treatment under test, wax and silane treatments were used for comparison. In total, three treatments were evaluated: the author's innovative biological treatment (T4), Cosmoloid H80 wax (TR), and Dynasylan® F8263 (T1). These were compared to coupons with no treatment (T0) for reference. The biological treatment was applied according to procedures developed at the laboratory of microbiology using a specific strain of *B. bassiana*. Several application methods (labelled A to G) were assessed based on the use of either spores or mycelium of *B. bassiana* and different nutrient sources; Dynasylan F8263 was applied until saturation (5 times); Cosmoloid wax H80 was applied under the supervision of a conservator and restorer.

All coupons were exposed to the urban marine environment of the ISMAR SMS site in Genoa Harbour from December 2011. The monitoring is ongoing and data after three, six and twelve months has been collected. The overall period of exposure is planned to be 18 months.

Colourimetry

Measurements of the colourimetric coordinates in the space CIEL*a*b* were performed in order to evaluate possible chromatic alterations due to the application of superficial treatments and/or weathering. A Minolta d2600 and an Avantes HL-2000 were used, with illuminant D65 and a 10 degree observer, using CIELab 1976 colour space. On each coupon, three to four measurements were carried out at different spots and the mean value calculated. Colour differences have been computed as Equation 1:

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Values lower than 1 are considered imperceptible, while $\Delta E^*>5$ is considered clearly perceivable.

Electrochemical impedance spectroscopy (EIS)

EIS measurements were performed with a specially designed contact probe (ST15). The probe area was 1.77 cm^2. A commercial cleaning cloth soaked with a mineral water (electrical conductivity 320 μS/cm, pH=7.9) was fixed to the contact cell, and the system obtained then leant on the surface to be measured. The cloth was immersed in mineral water for 120 min before being fixed to the contact cell. The EIS spectra acquisition started around 30 min after the cell was leant on the measurement area in order to stabilise the open circuit potential. EIS measurements were made with a Gamry REF600, with Framework/EIS300 V5.3 software. Spectra with 10 points per decade were acquired in potentiostatic mode with 10 mV AC signal level at open circuit potential. Measurements were acquired in the frequency range 100 KHz - 10 MHz. For each system under examination, two to seven spectra were measured in order to check homogeneity and repeatability of results. At first, measurements in the centres of coupons of a series were made, and further measurements were added when needed to obtain a statistically meaningful set of data. Soon after each measurement a digital photo of the sample with the wet footprint of the EIS contact probe was recorded with a VEHO VMS-004 USB Microscope by MicroCapture software; samples were photographed on graph paper in order to calibrate the picture and use the MicroCapture software to determine the wet footprint area for each measurement.

FTIR microscopy

All coupons were analysed superficially and non-destructively, without any type of preparation; they were simply positioned on the FTIR microscope stage. Reflectance spectra were acquired in the range of 4,000 - 650 cm^{-1} on a Biorad excalibur spectrometer connected to a Varian UMA500 microscope, fitted with a MCT (Mercury Cadmium Telluride) detector cooled by liquid nitrogen. Measurements were made with the microscope in reflection mode, using a 15x objective and a tube factor of 10x. Single point reflectance measurements were performed with aperture of 250 x 250 μm by recording a total of 64 scans and averaging the resulting interferogram, with a resolution of 4 cm^{-1}. Data collection and post-run processing were carried out using the Varian resolutions ProTM software. Four points were measured for each coupon.

Results and discussion

Optimisation tests

Visual observations first allowed us to discriminate between the different T4 application protocols. Coupons treated according to protocols T4B, T4D, T4E and T4G presented the most homogeneous surface after treatment and were further characterised with colour and EIS measurements, while the others (T4A, T4C and T4F) showed a wide chromatic inhomogeneous variation of their surface (data not shown) and were discarded. Coupons treated with T4B showed a much larger colour variation amongst the untreated patinas (Fig. 1).

Further analysis by electrochemical impedance spectroscopy allowed the evaluation of the performance of the copper-oxalates patinas which had been obtained (Fig. 2).

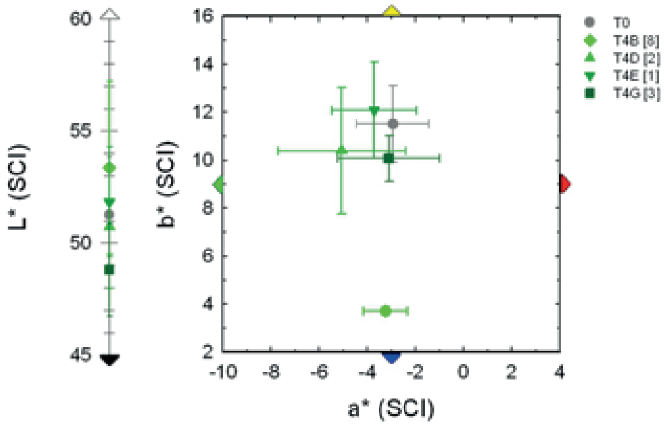

Figure 1: Colour comparison for different application methods used for the biological treatment. Values in square brackets in the legends are the ΔE values respect to T0.*

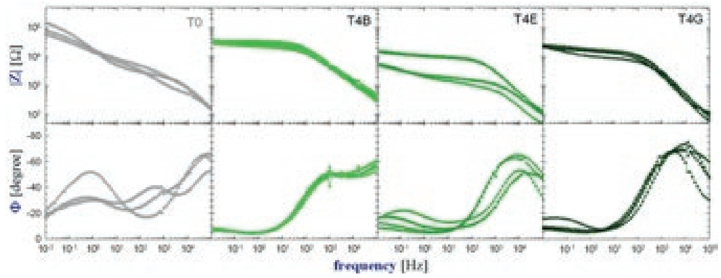

Figure 2: EIS spectra on copper urban natural coupons without treatment (T0) and with the biological treatment according to different application methods.

The better results were obtained for T4B, followed closely by T4G, while T4E presented less effective and more dishomogeneous results. Coupons exposed for the ageing test and treated according to protocol T4G showed less colour variation. Nonetheless T4B also may be considered for future applications. All protocols presented comparable phase curves, indicating that the same chemical process were involved in the formation of copper oxalates, and thus one might conclude that the application mode does not interfere on the behaviour of *Beauveria bassiana*.

Starting point

FTIR spectra of coupons treated with Dynasylan F8263 (T1) showed characteristic absorbance bands (asymmetric stretching Si-O) at 1251 and 1223 cm^{-1}. Those treated with the cosmoloid wax (TR) presented typical aliphatic chain vibrations, in particular at 2937 and 2857 cm^{-1}. Finally, the coupons treated with *Beauveria bassiana* had O-C-O bending at 1364 and 1320 cm^{-1} (Fig. 3).

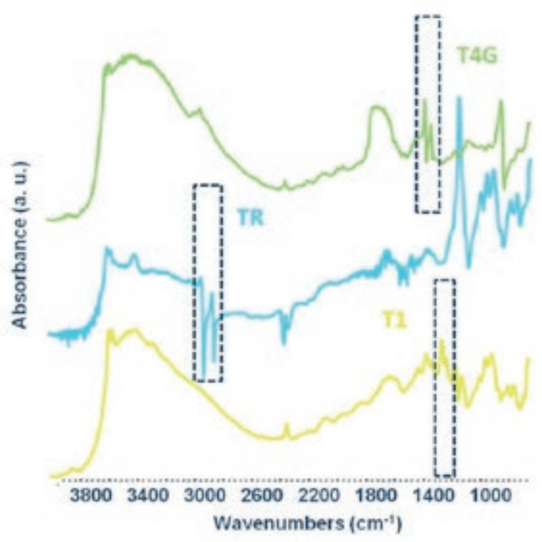

Figure 3: Reflectance FTIR spectra obtained from treated CUN coupons (urban patina) at zero months of exposure. QQ coupons (marine patina) presented the same characteristics bands for the different treatments under study (data not shown).

Comparing the colour measurements before and after treatment, the optical changes (ΔE) were clearly perceptible (ΔE ≥5) for TR and just perceptible (ΔE* ≥2) for T1 and T4G (Table 1).*

Treatment	ΔE* QQ coupons	ΔE* CUN coupons
T1	5	3
T4G	3	4
TR	12	43

Table 1: Colour change ΔE with respect to untreated samples after application of treatments at zero months of exposure. QQ coupons= marine patina; CUN= urban patina.*

The high impedance modulus values |Z| for TR indicated an excellent level of protection achieved (Fig. 4), meaning that the wax acts as a physical barrier, blocking pores. However, these values were quite different to those obtained for T1 and T4G. These two treatments have a different protection mechanism from wax, making their comparison difficult. Some protection may also be provided by T1 as its impedance values were always greater than those of the untreated patinas (Fig. 4). For T4G, the |Z| values were lower than the original patina present on the coupons and this is due to the fact that the biological treatment is based on an acidic process with release of oxalic acid by *Beauveria bassiana* (Fig. 4).

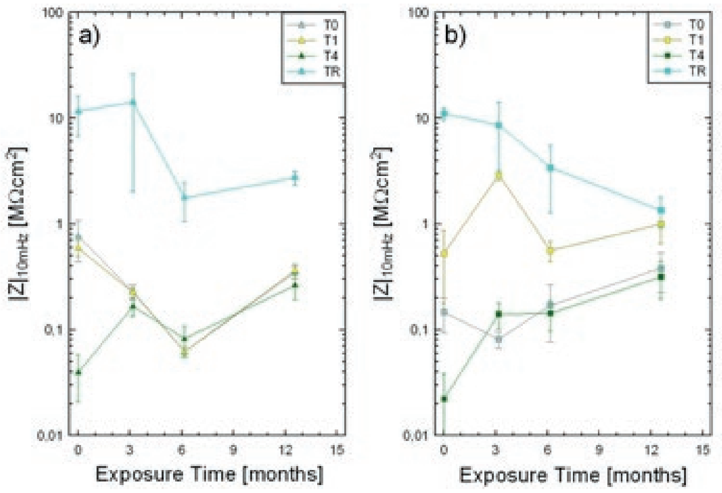

Figure 4: Average $|Z|_{10MHz}$ values measured at zero, three, six and twelve months of exposure on QQ and CUN samples treated with none (grey), T1 (yellow), TR (blue) and T4G (green).

Monitoring

EIS measurements were performed for QQ and CUN coupons at three, six and twelve months to monitor the long-term behaviour of the treatments (Fig. 4). As already reported for similar protective systems, the protection performance of TR decreased in the first months of exposure. This aspect underlines the importance of performing natural ageing tests over a rather extended period. After three months' exposure, T1 showed a slight impedance decrease which became accentuated after six months to reach similar impedance values as the untreated patina at twelve months. On CUN coupons, better protection seems to have been achieved by T1 with a higher impedance modulus value. T4G had completely different protection behaviour: the impedance values were very low at the beginning. However, after six and twelve months' exposure, the protection performance increased and became similar to the bare patina. These results suggested a stabilisation in progress, and a complete data interpretation will be obtained over the next months. Samples with TR presented more significant changes of aspect, having become whitish and stained. The untreated sample patina showed no evidence of colour changes. The surface changes visually observed at the beginning for the samples treated with T4G disappeared, and these patinas presented a green and relatively more uniform aspect (data not shown).

Conclusions

This paper presents an innovative treatment in the field of the conservation of ancient metallic artworks. Using properties of a specific fungal strain, namely *Beauveria bassiana*, existing corrosion products were converted into copper oxalates. These could provide treated objects with long-term protection without aesthetic alteration. For the first time, the performances of the biopatina were monitored and compared with materials traditionally used in conservation and restoration. Naturally aged coupons of copper were treated, and very promising results were obtained after a twelve month period of ageing. In particular, the biopatina showed a lower colour variation, and a corrosion stabilisation process seemed to be in progress. In order to confirm these results, monitoring will be extended to 18 months. The successful submission to a JERICO grant (VII FP, Contract 262584) on the application of electrochemical techniques to the evaluation of coatings for cultural heritage will allow access to the ISMAR facility to conclude the ageing procedure.

Based on the outcome of this study, the authors will be able to develop a standardised product that could be transferred into real praxis. In order to develop a user-friendly kit, trial tests on real objects and further tests for the appearance, resistance to corrosion and cohesion of the biopatina onto the object's surface will be carried out.

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